#944

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8885

OCT 19 1973

Application for Research Grant

(Use extra pages as needed)

Date: Oct. 1, 1973

1. Principal Investigator (give title and degrees): David Glick, Ph.D., Director
Center for Histochemical Research

2. Institution & address: Stanford Research Institute
Life Sciences Division
333 Ravenswood Avenue
Menlo Park, CA 94025

3. Department(s) where research will be done or collaboration provided: Life Sciences Division

4. Short title of study: Quantitative Histochemical Studies on Lung Cancer Induced by Carcinogenic Agents

5. Proposed starting date: November 1, 1973

6. Estimated time to complete: 1 year. Renewal to be dependent on mutual desire of both parties.

7. Brief description of specific research aims:

(1) Quantitative determination of cyclic AMP (cAMP) in cytologically defined samples of mouse lung tumors (adenomas) induced by benzpyrene and squamous cell carcinoma induced by 3-methylcholanthrene. Changes in cAMP concentration with correlation to the cytology will be followed as a function of malignant development and compared with appropriate nonmalignant controls.

The aim is to clarify to what degree these carcinogens can affect the cAMP control in lung cell function during carcinogenesis.

(2) Parallel study of benzpyrene and 3-methylcholanthrene hydroxylase activity in the same material. This will first require adaptation of the method for determination of the specific hydroxylase activity to the microscale needed for the small samples.

The aim is to clarify to what degree this enzyme, which is responsible for the first step in detoxification and elimination of the carcinogen, may undergo change in the induced carcinogenesis, possibly in an adaptive mechanism for defense against the action of the carcinogens.

10035513

Source: https://www.industrydocuments.ucsf.edu/docs/ykdm0000

8. Brief statement of working hypothesis:

(1) Involvement of cAMP in cell membranes with malignant development is now established; e.g., it has been shown recently that cAMP suppresses tumorigenicity of human cancer cells (Smith and Handler, Res. Commun. Chem. Pathol. Pharmacol. 5, 863, 1973; Fed. Proc. 32 (3), Pt. 1, Abstr. 2226, 1973), that cAMP levels are lower in cancer cells than in normal cells, and that cAMP is critically involved in cell-to-cell contact effects and their alterations in cancer (Emmelot, European J. Cancer 9, 319, 1973).

Accordingly, it is important to clarify the cAMP role in carcinogenesis in lung as induced by benzpyrene and 3-methylcholanthrene.

(2) Detoxification and excretion of benzpyrene and 3-methylcholanthrene are involved in the physiological defense to the action of the carcinogens; if there were an adaptive increase in the specific hydroxylase acting on these compounds to promote their transformation and elimination, this would oppose their influence in inducing lung tumors. It is essential to know to what degree the lung enzyme is affected by the hydrocarbons and how the enzyme activity changes in carcinogenesis.

9. Details of experimental design and procedures (append extra pages as necessary) The proposed research will be conducted in collaboration with Dr. Arthur Furst, Institute of Chemical Biology, University of San Francisco, who will provide the tissue from material he is already producing under a grant from the Council for Tobacco Research - U.S.A. Thus, additional information could be obtained from his material without interfering in any way with Dr. Furst's work, since the quantities of tissue needed in the quantitative histochemical studies proposed are very small.

Dr. Furst will provide lung tumor tissue from his genetically selected mice (C57Bl/6/J, C57Bl/6/Cum, dba/J, NIH-Swiss/MBA), in which the hydrocarbon-induced tumors have been formed, and control tissue samples from untreated mice, from nontumor regions of the lungs with tumors, and from corresponding benign lung tumors. He will also provide the histological examination and diagnosis of the tissues used. Each sample will be divided, one part for histological preparation and diagnosis by Robert Kovatch, D.V.M., and the other immediately fresh-frozen and delivered for analysis by microchemical procedures of quantitative histochemistry.

Samples will be prepared for measurement by cryostat sectioning of the fresh-frozen material, and the individual microtome sections will be collected in separate small glass tubes for chemical analyses, with an adjacent section to each one used for analysis taken for histological staining and diagnosis.

The cAMP will be measured by the fire-fly luminescence method as adapted by Orenberg and Glick (J. Histochem. Cytochem. 20, 923, 1972) for analysis of microtome sections of tissue, using improvements and simplifications more recently developed in our laboratory.

Depending on which proves most feasible for the present purpose, the specific hydroxylase activity will be measured by an adaptation of the fluorescence method described by Wattenberg et al. (Cancer Res. 28, 934, 1968), Nebert et al. (J. Biol. Chem. 243, 6242, 1968; Cancer Res. 29, 1763, 1969), or Kouri et al. (J. Nat. Cancer Inst. 49, 993, 1972).

Measurement of the amount of sample analyzed for calculation of concentration will be the wet weight (to permit comparison with certain published data) and the protein content (more analytically reliable and also used in certain published data). The latter will be measured by the bromsulfalein-binding method for protein, in use in the laboratory of the principal investigator for many years (Glick, Quantitative Techniques of Histo- and Cytochemistry, Vol. II, pp. 148-150, 155-160, Wiley - Interscience, N.Y., 1963).

100354139

Source: https://www.industrydocuments.ucsf.edu/docs/ykdm0000

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

A laboratory (644 ft^2) is available at Stanford Research Institute, Life Sciences Division (Building 28, Room Nos. 202 and 203), for the quantitative histochemical work.

11. Additional facilities required:

The aspect of the investigation that involves the collaboration of Dr. Furst will be conducted in his laboratory, which also has adequate animal and histology facilities (already site-visited by CTR personnel).

12. Biographical sketches of investigator(s) and other professional personnel (append):

Appendices 1, 2, 3.

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

Appendix 4, plus reprints.

10035145140

Source: https://www.industrydocuments.ucsf.edu/docs/ykdm0000

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount Salaries	Benefits	Total
David Glick, Ph.D., Principal Investigator	25	7,332	1,906	9,238
Dorothy von Redlich, Ph.D., Res. Assoc.	50	8,889	2,311	11,200
Yoshinao Katsumata, M.D., Res. Assoc.	75	10,672	2,775	13,447
Research Asst.-to be recruited	50	3,733	971	4,704
Technical				
Secretary, to be recruited	25	1,793	466	2,259
Dishwasher, Lab. Helper	25	1,603	417	2,020
		34,022	8,846	42,868

Sub-Total for A 42,868

B. Consumable supplies (by major categories)

Chemicals	1,200
Lab. Ware	800

Sub-Total for B 2,000

C. Other expenses (itemize)

Publications, Drawings, Photos, and Reports	400
Upkeep of Instruments	400
Miscellaneous	500
Travel-equiv. of 1 East Coast trip for 2 investigators	1,200
Sub-Total for C	2,500

Running Total of A + B + C 47,368

D. Permanent equipment (itemize)

Luminometer (du Pont)	6,100
Sub-Total for D	6,100

E. Indirect costs (15% of A+B+C) E 7,105

Total request 60,573

. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	Although anticipated as a 3-year project, the budget for the first year only is					
Year 3	given because future budgets would be determined by requirements made apparent					

10035545141

Source: https://www.industrydocuments.ucsf.edu/docs/ykdm0000

15. Estimated future requirements: (Continued)

in the first year. Renewal for subsequent years of the grant to depend on mutual desires of both parties.

100345142

Source: https://www.industrydocuments.ucsf.edu/docs/ykdm0000

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Quantitative Histochemistry of Gastric Acid Secretion	NIH - submitted	169,102	5/1/74 - 4/30/77

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name David Glick

Signature David Glick Date Oct. 1, 1973

Telephone 415 (Area Code) 326-6200 (Number) 2116 (Extension)

Checks payable to

nford Research Institute

Mailing address for checks

Life Sciences Business Office
Stanford Research Institute
333 Ravenswood Ave., Menlo Park, CA 94025

Responsible officer of institution

Typed Name Philip J. O'Donnell

Title Manager, Contract Administration

Signature Philip J. O'Donnell Date Oct. 15, 1973

Telephone 415 vc (Area Code) 326-6200 (Number) 2482 (Extension)

100354143

Source: https://www.industrydocuments.ucsf.edu/docs/ykdc0000